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Seasonal and Photoperiod-Induced Changes in Serum Prolactin and Pituitary Responsiveness to Thyrotropin-Releasing Hormone in the Mare (42455)

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Abstract. Experiments were conducted in the horse mare to study the effects of photoperiod and season on serum prolactin and pituitary responsiveness to thyrotropin-releasing hormone (TRH). Increasing the photoperiod to 16 hr light:8 hr dark beginning in December (Experiment 1) and September (Experiment 2) increased serum prolactin, but the rate of increase was greater when the photoperiod treatment was initiated in September. In addition, TRH-induced prolactin secretion was found to be affected by season, in that pituitary secretion (net increase in prolactin and total prolactin secreted) was significantly greater in June compared with that of January (Experiment 3). These data suggest that in the mare, photoperiod plays an important role in controlling circulating levels of prolactin, but that in addition to photoperiod, other seasonally related factors, such as temperature, are involved in modulating the seasonal rhythm of serum prolactin.

Serum concentrations of prolactin in the mare vary relative to season, with highest concentrations occurring from June through August and lowest from November through February (1). Results from other domesticated species (e.g., sheep (2), cattle (3)) have shown that a stimulatory photoperiod provided to animals during the winter months in the northern hemisphere increases circulating concentrations of prolactin. Furthermore, the capacity of the pituitary to secrete prolactin in response to thyrotropin-releasing hormone (TRH) varies with the photoperiod and parallels changes in basal prolactin levels (cattle (4), deer (5), sheep (6)).

Little information, however, is currently available from the horse concerning either the effects of photoperiod manipulation on serum prolactin, or seasonal effects on TRH-induced prolactin secretion. Therefore, the studies outlined herein were designed to investigate these aspects in the nonpregnant horse mare.

Materials and Methods. Standardbred mares, ages 5 to 20 years, were fed a ration (adjusted to maintain body weight) consisting of mixed hay, a commercial sweet feed (Agway, Bordentown, NJ) plus seasonal pasture, when available. Prior to the start of an experiment, mares were housed under ambient light and temperature conditions in an exercise lot or on open pasture. All mares used in these experiments were well accustomed to being handled and to having blood sampled by jugular venipuncture.

The first experiment was initiated in December, at the time of the winter solstice in the Northern hemisphere (photoperiod for New Brunswick, NJ at winter solstice is 8.3 hr light:15.7 hr dark; mean weekly temperature at the start of experiment was 5.1°C; Fig. 1). Mares which had previously been determined to be seasonally anestrous were randomly divided into two treatment groups, a repetitive 16-hr light:8-hr dark (16L:8D) photoperiod treatment group (n = 6 mares) or an ambient light treatment group (n = 6 mares). Mares exposed to 16L:8D were allowed access to an exercise lot from 0800 to 1600 hr, then were housed individually in light-tight box stalls and exposed to cool-white fluorescent light (General Electric, Watt-Miser; approximately 1000 lux at head level) from 1600 to 2400 hr. The temperature within the animal facility was not controlled, and followed the normal seasonal pattern for New Jersey. Ambient light-treated mares were kept outside in exercise lots throughout the experiment. Blood samples (8 ml) were drawn by jugular venipuncture (between the hours of 1000 and 1200) prior to the start of the experiment, and every Monday and Friday thereafter for a total of 10 weeks. Serum was collected and stored at −20°C until assayed for prolactin.

A second experiment was initiated in mid-September when the length of the ambient photoperiod was 12.4 hr and the mean weekly temperature was 17.5°C (Fig. 1). Cycling mares were randomly allotted to either an

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ambient light treatment (n = 7) or a repetitive 16L:8D photoperiod treatment (n = 4). The housing of animals and details of light treatments were as described above. Blood samples were drawn prior to the start of the experiment, and on Mondays and Fridays (between 0900 and 1200 hr) for 7 weeks thereafter. Serum was collected and stored at −20°C until assayed for prolactin.

A final experiment was conducted to assess seasonal changes in the ability of the pituitary to secrete prolactin in response to TRH. Cycling mares were injected (without regard to stage of the estrous cycle), iv, with either 50 μg TRH (Peninsula Laboratories, Belmont, CA) or vehicle (2.0 ml 1% sterile saline) in January (photoperiod was 8.8 hr light; temperature, −1°C) (n = 4 vehicle-injected, 5 TRH-injected) or June (photoperiod, 15.1 hr light; temperature, 21°C) (n = 4 vehicle-injected, 7 TRH-injected). Blood was drawn (5.0 ml) by jugular venipuncture 30 min and immediately prior to injection, then 5, 15, 30, 45, 60, 90, 120, 180, and 240 min following injection. Serum was collected and frozen at −20°C until assayed for prolactin.

The radioimmunoassay for equine prolactin was conducted as previously described (1). All samples from an experiment were assayed in duplicate within the same assay. The mean within- and between-assay coefficients of variation for the three experiments reported were 8.3 and 11.1%, respectively. Prolactin data from the first two experiments were analyzed by regression analysis, and the slopes of the

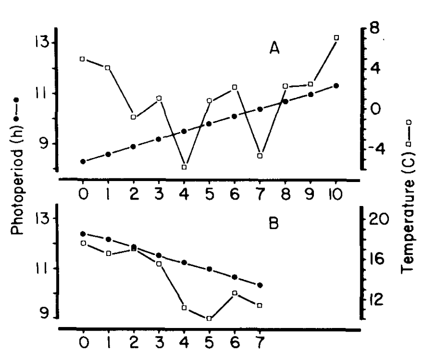

FIG. 1. Mean weekly ambient photoperiod and temperature from December to February (A, Experiment 1) and September to November (B, Experiment 2) for New Brunswick, New Jersey (40° 30′ north latitude).

linear regression equations were compared as described by Zar (1974). Pretreatment concentrations between photoperiod treatments within an experiment for all three experiments were compared by t test. Serum prolactin concentrations following injection with TRH or vehicle were analyzed by a split-plot in time analysis of variance (ANOVA) (7). In addition, because preinjection concentrations of prolactin between months (January versus June) were determined to be significantly different, data were also expressed as the net increase in prolactin (i.e., hormone concentration postinjection minus preinjection value, in ng/ml). Total prolactin secreted for each treatment group was calculated by determining the total area from the series of trapezoids formed from each time interval versus the net increase in prolactin. The baseline utilized for calculating the area under the curve was the mean prolactin concentration for samples drawn −30 min and immediately preceding the TRH or vehicle injection. The peak values, time of peak, and total prolactin secreted after TRH or vehicle injection for both months were compared by a one-way analysis of variance, and significant interactions were partitioned by the Newman–Keuls multiple range test.

Results. Results from the study initiated at the time of the winter solstice showed that serum prolactin increased subsequent to the initiation of the 16L:8D treatment (regression equation, $Y = 0.005\,X + 1.24$, $r = 0.601$, $P = 0.002$), whereas prolactin concentrations in the ambient light group remained unchanged throughout the 10-week sampling interval ($Y = 0.000\,X + 0.93$, $r = -0.146$, $P > 0.50$). The slopes of the two regression equations were significantly different ($P < 0.05$). Pretreatment concentrations of prolactin were not different between the 16L:8D photoperiod treatment group and the ambient light-treated group (0.91 ± 0.10 ng/ml vs 1.05 ± 0.13 ng/ml, $P > 0.20$) (Fig. 2).

Prolactin concentrations in mares housed under ambient light conditions from September to November decreased ($Y = -0.011\,X + 2.15$, $r = -0.610$, $P = 0.015$), whereas levels in mares exposed to a constant 16L:8D photoperiod beginning in September increased ($Y = 0.019\,X + 1.96$, $r = 0.836$, $P < 0.001$) (Fig. 3). Pretreatment concentrations were similar between the 16L:8D and the ambient light-treated groups (1.90 ± 0.22 and 2.36 ± 0.42

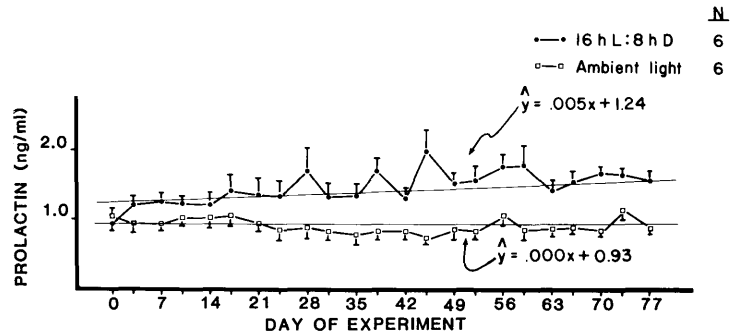

FIG. 2. Serum concentrations of prolactin (ng/ml ± SEM) from mares exposed to either increasing ambient photoperiod or a continuous 16-hr light:8-hr dark photoperiod beginning at the time of the winter solstice.

ng/ml, respectively, $P > 0.20$); however, the slopes of the two regression equations calculated from concentrations obtained during the treatment interval were determined to be different ($P < 0.05$).

Thyrotropin-releasing hormone induced the release of prolactin in mares exposed to short photoperiod (mares injected in January; $P < 0.001$ by ANOVA for repeated measures) and long photoperiod (mares injected in June; $P < 0.001$); however, the peak and the total prolactin released in response to TRH were significantly greater in mares exposed during the long photoperiod (Table I). Mean concentrations of prolactin were unchanged throughout the sampling interval (as indicated by split-plot in time ANOVA) in both groups of vehicle-injected controls. As expected, preinjection concentrations of prolactin were higher in mares sampled in June (overall mean, 3.32 ± 0.15 ng/ml, $n = 11$) compared with those sampled in January (0.94 ± 0.03 ng/ml, $n = 9$; $P < 0.01$).

Discussion. Serum prolactin in mares exposed to a 16L:8D photoperiod beginning at the winter solstice was increased over pretreatment levels by an increment of 0.64 ng/ml (or a 70.3% over pretreatment concentrations) at the end of the 10-week treatment period. Although this increase in prolactin in response to 16L:8D was somewhat less than that previously found after 12 to 14 weeks of 16L:8D (a 150% increase compared with ambient light-treated controls) or the skeleton photoperiod, 10L:8D:2L:4D, (a 111% increase compared with ambient controls) beginning

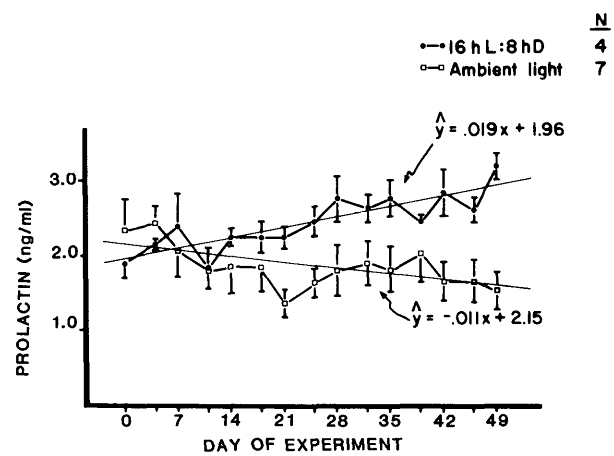

FIG. 3. Serum concentrations of prolactin (ng/ml ± SE) from mares exposed to either decreasing ambient photoperiod or a continuous 16-hr light:8-hr dark beginning in September.

TABLE I. SERUM PROLACTIN RESPONSE TO AND iv INJECTION OF VEHICLE OR 50 μg THYROTROPIN-RELEASING HORMONE (TRH) IN JANUARY OR JUNE

Month	TRH	*N*	Preinjection concentration (ng/ml + SE)	Peak		Area[a] (ng/ ml × min + SE)
				Δ ng + SE	Time to: (min + SEM)	
January	0	4	0.97 + 0.04[b]	—	—	−26.78 + 42.96[b]
	50 μg	5	0.93 + 0.04[b]	2.12 + 0.82[b]	24.0 + 3.7	151.65 + 71.98[c]
	Overall mean ± SE		0.94 + 0.03			
June	0	4	3.11 + 0.11[c]	—	—	0.84 + 50.99[b]
	50 μg	7	3.44 + 0.17[c]	5.37 + 0.48[c]	21.4 + 4.5	534.74 + 37.21[d]
	Overall mean + SE		3.32 + 0.15			

[a] Integrated area under curve for prolactin concentrations after vehicle or TRH injection minus preinjection concentration.

[b–d] Values not followed by common superscript within a column are significantly different ($P < 0.05$).

in early December (8), the difference in magnitude of increase may be related to the length of photoperiod treatment. The lack of change in serum prolactin from December through February in ambient light-treated mares was not unexpected in view of the lack of change in the annual profile of serum prolactin during this period, as recently described (1). Similarly, mares exposed to a 16L:8D photoperiod beginning in September responded with a net increase of 0.70 ng prolactin/ml serum (a 36.8% increase compared to pretreatment concentrations) over the 7-week treatment period, whereas concentrations decreased by 0.68 ng/ml (a 71.1% decrease) in ambient light-treated mares. This decrease in serum prolactin in mares exposed to ambient light from September to November was more gradual than previously reported (1). At this time we would suggest that the difference in results from the two studies represents a year-to-year variation in the prolactin profile, which might be modulated by seasonal temperature.

It is of interest to note, however, that in neither experiment did the magnitude of increase nor the highest values obtained for serum prolactin in response to 7 or 10 weeks of a 16L:8D photoperiod approach values comparable with maximal levels observed during the annual rhythm. Highest annual concentrations of prolactin (in July) were found to exceed 3 ng/ml serum, which, when compared with concentrations found during December, represents an overall seasonal related increase of greater than 200% (1). It is possible that this attenuated response to an artificial stimulatory photoperiod either during the winter months (Experiment 1) or at a time when prolactin concentrations are naturally decreasing (Experiment 2) could be due to the length of photoperiod treatment (10 or 7 weeks, compared with the annual rhythm in which increasing concentrations occur over a 6-month interval). However, results from studies with prepubertal bulls show that serum prolactin increased by greater than sixfold over a 5-week treatment period following an increase of photoperiod from 8L:16D to 16L:8D (4), while in sheep, prolactin levels increased by three- to fivefold in sightly longer than a 5-week period of exposure to 16L:8D (9).

Alternatively, the attenuated response could be due to a seasonally related change in the ability of the pituitary to secrete prolactin. Therefore, the third experiment was conducted to compare pituitary responsiveness with a known prolactin secretagogue (TRH) during January and June. Results of this experiment clearly show that TRH responsiveness is greater in June than in January (Table I). Although this present experiment was conducted in cycling mares without regard to stage of the estrous cycle, we have previously determined that serum concentrations of prolactin do not change relative to day of ovulation (1). Furthermore, the response in prolactin secretion following injection of TRH is not different in diestrous (Days 9–10 of cycle) compared with estrous (Days 18–19) mares (Johnson, unpublished data). These data, which indicate that a long photoperiod enhances pituitary responsiveness to TRH, are consistent with results from other domesticated species, including sheep (6), cattle (4, 10), and deer (5). However, in addition to photoperiod-related changes, there have been reported changes in pituitary sensitivity to TRH and the prolactin response to long photoperiod which can be directly attributed to ambient temperature. For instance, Tucker and Wettemann (11) have previously shown that in heifers, TRH-induced prolactin secretion increases as the ambient temperature increased. Furthermore, Peters and Tucker (3) determined that the overall increase in serum prolactin in response to ambient or a 16L:8D photoperiod was directly related to the ambient temperature. In the present experiments, where no attempt was made to control ambient temperature, the rate of increase in serum prolactin in response to a 16L:8D photoperiod was greater during the fall (range of ambient temperature for the 7-week period, 19 to 4°C; slope of prolactin curve = 0.019) than in winter (range for the 10-week period, −6 to 8°C; slope of prolactin curve = 0.005, $P < 0.05$). These data suggest that ambient temperature may play a significant role in controlling prolactin secretion.

In summary, results from the present experiments show that the transfer of mares from a short (in December) or decreasing (in September) ambient photoperiod to a long photoperiod (16L:8D) results in an increase in serum prolactin. In addition, the ability of the pituitary to secrete prolactin is influenced by season, in that a challenge with TRH during the summer results in a significantly greater release of prolactin compared with that during the winter months. We would suggest that in addition to photoperiod, ambient temperature may be involved in modulating the annual rhythm of prolactin secretion in the mare.

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